Application No. 10/758,401
Restriction Requirement dated August 29, 2006
Response to Restriction Requirement mailed December 5, 2006

Listing of the Claims:

This listing of the claims replaces all the prior versions and listings of the claims.

1. (Cancelled)

2. (Currently Amended) A method of amplifying ~~the~~ a hairpin structure comprising converting a double stranded nucleic acid into a hairpin structure, wherein the double stranded nucleic acid contains at least one sequence of interest, and is referred to as the template nucleic acid, comprising either (1) ligating a first single stranded nucleic acid to a first end of the upper strand of the template nucleic acid, and ligating a second single stranded nucleic acid which is non-complementary to the first single stranded nucleic acid to the first end of the lower strand of the nucleic acid; or (2) ligating a cap of single stranded nucleic acid to both the upper strand and the lower strand of the first end of the template nucleic acid, wherein said cap contains a sequence about midway in the cap, and that cannot be amplified by polymerase chain reaction (PCR), and wherein the nucleic acid bases on either side of this sequence are not complementary to each other; and further comprising ligating a cap of single stranded nucleic acid to both the lower strand and the upper strand of the second end of the nucleic acid, such that the upper strand and the lower strand of the second end are contiguous, creating the final hairpin structure ~~of claim 1~~, and further comprising performing polymerase chain reaction with a first primer that binds to at least a portion of the upper single stranded non-complementary region, and a second primer that binds to at least a portion of the lower single stranded non-complementary region.

3-5. (Cancelled)

6. (Original) A method of amplifying a nucleic acid sequence of interest which generates a PCR-amplified product which is substantially free of polymerase-induced errors, comprising:

(a) converting the sequence of interest into a hairpin DNA structure;

(b) amplifying the hairpin DNA using PCR with a first primer that binds to at least a portion of the upper single stranded region, and a second primer that binds to at least a portion of the lower single stranded region;

(c) converting the PCR products into hairpin structures by a method which induces denaturation followed by sudden renaturation;

(d) identifying hairpins containing polymerase-generated nucleotide changes, insertions, and deletions, via the resulting mismatched bases comprising gaps in binding, and

(e) removing such hairpin DNAs containing polymerase generated mismatched nucleotides, and collecting the DNA that contains no mismatches.

7. (Original) The method of claim 6, wherein the method which induces denaturation followed by sudden renaturation is selected from the group consisting of (a) heat denaturation followed by rapid cooling, (b) addition of sodium hydroxide followed by sudden neutralization of the solution, and (c) addition of formamide followed by sudden removal of formamide.

8. (Original) The method of claim 6, wherein the hairpin DNAs containing PCR-induced errors have a mismatch in the double stranded region and are separated from hairpin DNAs which do not contain PCR-induced errors by a method which recognizes DNA containing a mismatch.

9. (Original) The method of claim 8, wherein the method which recognizes DNA containing mismatches is selected from the group consisting of dHPLC-mediated fraction collection, denaturing gradient gel electrophoresis (DGGE), constant denaturant gel electrophoresis (CDGE), constant denaturant capillary electrophoresis (CDCE), and an enzymatic-based separation method.

10. (Original) The method of claim 9, wherein the enzymatic-based separation method is performed either in solution or bound to a solid support, and the enzyme is at least one enzyme selected from the group consisting of mismatch-recognition enzymes MutS, MutY, and TDG; Cel I; resolvases; endonuclease V; cleavases, and exonucleases.

11. (Original) The method of claim 6, wherein (a) during the course of amplification the polymerase-generated errors are converted to mismatches and remain as mismatches during each cycle of amplification, and (b) following the end of amplification all the polymerase-generated errors are in a mismatched structure while all the mutations are in a matched structure.

12. (Original) The method of claim 6, wherein one strand of the template nucleic acid is joined with a second, fully complementary nucleic acid strand such that the two strands are contiguous, and such that during amplification the polymerase copies both the upper strand of the template nucleic acid and the lower strand of the template nucleic acid in a single pass.

13. (Original) The method of claim 12, wherein the upper strand of the template nucleic acid is joined with the fully complementary lower strand of the template nucleic acid, such that during amplification the polymerase copies both the upper strand and the lower strand in a single pass.

14. (Original) A method of amplifying a nucleic acid sequence of interest which generates a PCR-amplified product which is substantially free of polymerase-induced errors, comprising:

(a) converting the sequence of interest into a hairpin DNA structure;

(b) amplifying the hairpin DNA using PCR with a first primer that binds to at least a portion of the upper single stranded region, and a second primer that binds to at least a portion of the lower single stranded region; wherein the concentration of primers are either equal to each other ('regular PCR') or unbalanced ('asymmetric PCR');

(c) identifying hairpins containing polymerase-generated nucleotide changes, insertions, and deletions, via the resulting mismatched bases comprising gaps in binding; and

(d) removing such hairpin DNAs containing polymerase generated mismatched nucleotides, and collecting the DNA that contains no mismatches.

15. (Original) A method of improving the fidelity of an assay that relies on a PCR-amplified nucleic acid template for at least one step of the assay, wherein the PCR-amplified nucleic acid template is generated using the method of claim 6.

16. (Original) The method of claim 15, wherein the assay is selected from the group consisting of mutation detection, mutation analysis, polymorphism detection, polymorphism analysis, microsatellite analysis, cloning, and protein functional analysis.

17. (Original) The method of claim 16, wherein the method of mutation or polymorphism detection is selected from the group consisting of PCR, PCR/RE/LCR, MutEx-ACB-PCR, RFLP analysis, and APRIL-ATM.

18-34. (Cancelled)